DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

March 13, 2003

FAP 2234
Natamycin

DUCOA
c/o Mr. Franklin Carter
1082 Duna Drive
Laramie, Wyoming 82072

Dear Mr. Carter:

We have completed our review of your food additive petition dated July 31, 2001. The petition seeks to amend Federal food additive regulations to permit the use of natamycin in broiler chicken feeds for the purpose of retarding the growth of *Aspergillus parasiticus* in the feed for up to 14 days. The petition is an amendment to one we filed for you on September 6, 1995, and in which you attempted to establish the safety and utility of natamycin when used in broiler chicken feeds to retard the growth of *Aspergillus parasiticus*, *Penicillium rubrum*, and *Fusarium moniliforme*.

The results of our review of the current petition are as follows:

Name and other pertinent information

We informed you in our letter of June 6, 1996, that we found several sections of your petition of September 6, 1995, to be satisfactory. The sections that we found to be satisfactory included those dealing with the name, chemical identity, chemical composition, properties, method of manufacture, method of analysis, and stability of natamycin.

Utility

You conducted three experiments in the laboratory to establish the utility of natamycin for retarding the growth of *Aspergillus parasiticus* in broiler chicken feeds for up to 14 days. All three experiments revolved around the use of a respirometer to measure the volume of gaseous exchanges in chambers attached to the respirometer. The first experiment was conducted with sterile, empty (no liquid medium, no feed, no spores) Erlenmeyer flasks to validate the proper functioning of the respirometer. The experiment was repeated once. In both cases, the respirometer was found to function adequately.

The second experiment was conducted to establish a correlation between the growth of *Aspergillus parasiticus* and the quantities of oxygen and carbon dioxide the fungus consumes and produces, respectively, during growth. In the experiment, spores of *Aspergillus parasiticus* were inoculated into a liquid medium in Erlenmeyer flasks and allowed to germinate and grow for 7 days while the flasks were connected to the respirometer. The experiment involved the use of 28 experimental flasks, and 8 control flasks. Four of the eight control flasks contained only the liquid medium (no spores). The other four were empty (no

medium, no spores). The cumulative amounts of oxygen consumed, and carbon dioxide produced, by germinating spores in each flask were recorded each day. At the end of each day, four flasks were disconnected from the respirometer and the mycelial mass in each separated from the liquid medium by filtration, and dried and weighed. The growth of *Aspergillus parasiticus*, as measured by daily changes in weight of the mycelial mass over 7 days, was compared to the amount of oxygen consumed or the quantity of carbon dioxide produced each day. The experiment was repeated once. In both cases, you established the existence of a direct correlation between the rate of gain of mycelial mass (growth) of *Aspergillus parasiticus* and the volume of oxygen consumed, or of carbon dioxide produced, by the fungus.

In the third experiment, the respirometer was used to evaluate the ability of natamycin to retard the growth of *Aspergillus parasiticus* in complete broiler chicken feeds for up to 14 days. A commercial broiler starter mash devoid of medications was sterilized by autoclaving. The sterile feed was divided into six lots of approximately 165 g. Sterile water, or an aqueous solution of a natamycin premix, was aseptically mixed into five of the six lots of feed to yield lots of feed containing natamycin at the levels of 0, 5.5, 11.0, 16.5, or 22 ppm. Each of the five lots was inoculated with enough spores of *Aspergillus parasiticus* to yield feeds containing ten to twelve thousand ($10 - 12 \times 10^3$) spores/g. The moisture content of each lot of feed was about 16.5 %. The sixth lot of sterile feed was left untouched (no water, no natamycin, no spores added) and served as the negative control. After about 4 days of incubation, approximately 30 g was extracted from each lot of feed and transferred into four Erlenmeyer flasks (four replicates per lot). The 24 flasks and four empty flasks (no water, no natamycin, no spores, no feed) were connected to the respirometer, and the cumulative amounts of oxygen consumed, or carbon dioxide produced, measured at 8 hour intervals for 14 days. The experiment was repeated once.

In both cases, you were able to establish that oxygen consumption or carbon dioxide production, respectively, were significantly reduced or increased in flasks containing natamycin at 11.0 ppm or higher. Based on the results of your second set of experiments, in which you established a direct correlation between growth of *Aspergillus parasiticus* and the quantities of oxygen or carbon dioxide the fungus consumes or produces, it is reasonable to conclude that a natamycin premix (composed of calcium carbonate, natamycin and lactose) will retard the growth of *Aspergillus parasiticus* in broiler chicken feeds when the amount of premix added to the feed is enough to provide natamycin at levels of 11.0 ppm or higher.

Unfortunately, your experiments did not contain the type of controls that would enable one to determine whether or not the effects observed can be attributed solely to natamycin, or to natamycin only when it is combined with lactose and calcium carbonate as done in your experiments. This should not adversely affect your petition provided the enabling regulation is written to reflect the need for natamycin to be marketed and used in the form of a premix containing specified quantities of natamycin, calcium carbonate, and lactose. If your desire is to have us write a regulation for natamycin by itself, you will need to repeat your third experiment to include a control involving the use of the natamycin premix without natamycin, or provide us with published data or other reliable information that demonstrate the anti-fungal activity of natamycin itself as well as the lack of anti-fungal activity of

calcium carbonate and/or lactose at levels at which use of your product will cause them to be present in feeds. Please let us know your preference.

Also, in our letter of June 6, 1996, we expressed our concern about inconsistencies you reported about the final concentrations of natamycin in some of the feeds used in your field trials. We were particularly concerned about those instances in which the levels of natamycin were found, on analysis, to be significantly lower than the amount purported to have been added. You informed us during subsequent telephone conversations that the problem was caused by the addition of the natamycin premix to the feed at the same time as the addition of liquid components of the feed, but have not provided any documentation to that effect. The issue needs to be resolved, and you can do that by providing us with language that describes the proper way to mix your natamycin premix into broiler chicken feeds to ensure a final concentration of natamycin in the feed that is the same as that intended.

Our statisticians have additional comments that you might find useful for conducting similar experiments in the future. Please contact Dr. Jesse Meneses of our Biometrics Team (JMeneses@cvm.fda.gov) at 301-827-0229, if you are interested.

Human food safety

We informed you in our letter of June 6, 1996, that we found this section of your petition to be satisfactory and have no human food safety concerns at this time regarding the use of natamycin at 11 ppm in broiler chicken feeds.

Target animal safety

We informed you in our letter of June 6, 1996, that we found the section of your petition dealing with target animal safety to be satisfactory.

Environmental assessment

We informed you in our letter of June 6, 1996, that we have carefully considered the potential effects associated with the approval of natamycin as proposed, and determined that the manufacture and use of the product is not expected to have a significant impact on the human environment. Therefore, we said an environmental impact statement is not required, and prepared a finding of no significant impact (FONSI) for this action.

Proposed label; proposed regulation

Based on the information you have provided, and until you provide us additional information clarifying the roles of calcium carbonate and lactose, we suggest that you modify your proposed label along the following lines:

Name: Change "NSURB (Mold retardant)" to "NSURE."

Claim: Change "For the retardation of growth of *Aspergillus parasiticus* in broiler chicken feeds for up to 14 days" to "For use in retarding the growth of *Aspergillus parasiticus* in broiler chicken feeds for up to 14 days."

Listing of ingredients: Change "Contents" to "Ingredients", and delete the words "Active" and "Inert carriers." List the ingredients as calcium carbonate, natamycin, and lactose, specify their respective proportions in the premix (g/lb) as 432.708, 11.292, and 10.000 (or as 433, 11, and 10) and include their CAS numbers.

Directions for use: Delete "Recommended use level of 1 pound per ton of feed" and replace with "Directions for use: Mix 1 pound (0.45 kg) of NSURE into 1 ton of broiler feed." Add "xxxxxx" (i. e., the mixing language that we asked for), and the following statement "Use natamycin-treated feeds within 4 weeks of treatment."

Caution: Delete "Store in tight, well-closed, light-resistant container in cool dry area" and replace with "Caution: Store in a tightly closed, light-resistant container in a cool, dry place."

The expiration date for natamycin premix is one year from the date of manufacture.

With respect to your proposed regulation, we have the following suggestions based on information you have provided us so far: You current proposal calls for the following –

"The food additive, natamycin, may be safely used in broiler chicken feeds in accordance with the following conditions:

(a) The additive is stereoisomer of 22-[(3-amino-3,6,dideoxy-B-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid with the empirical formula $C_{33}H_{47}NO_{13}$.

(b) The additive shall conform to USP specifications.

(c) The additive is used or intended for use as a mold retardant for *Aspergillus parasiticus* in broiler chicken feed for a period of time up to 14 days.

(d) The level of use of the additive shall not exceed a concentration of 11 ppm in broiler chicken feed formulations.

(e) To assure the safe use of the additive, the label or labeling of the additive shall bear, in addition to other information required by the Act:

(1) The name of the additive

(2) Adequate directions to provide a final product that complies with the limitations prescribed in paragraph (d) of this section."

We suggest that the proposal be modified to read as follows:

"The food additive, natamycin (CAS number 7681-93-8), may be safely used in broiler chicken feeds in accordance with the following specifications:

(a). The additive is a stereoisomer of 22-[(3-amino-3,6,dideoxy-B-D-mannopyranosyl)oxy]-1,3,26 trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid with the empirical formula $C_{33}H_{47}NO_{13}$.

(b). The additive shall conform to USP specifications.

(c). The additive (as part of a premix composed of calcium carbonate, natamycin, and lactose) is used for retarding the growth of *Aspergillus parasiticus* in broiler chicken feeds for up to 14 days

(d). Each pound (454 g) of the premix shall contain 433 g of calcium carbonate, 11 g of natamycin, and 10 g of lactose. The premix shall be mixed into broiler chicken feed at the rate of 1 pound (0.454 kg) per ton of feed to provide natamycin at a level of 11 ppm. "xxxxxxxx" (the mixing language we asked for). Broiler feeds to which the natamycin premix is added shall be used within 4 weeks of addition of the premix.

(e). To assure the safe use of the additive, the label or labeling of the additive shall bear, in addition to other information required by the Act, the following:

1. The name and CAS number of the additive.

2. A listing of ingredients consisting of calcium carbonate, the additive, and lactose and their proportions in the premix as prescribed under paragraph (d) of this section.

3. Adequate directions for use to ensure a broiler chicken feed that is in compliance with the limitations prescribed in paragraph (d) of this section.

4. An appropriate cautionary statement, "Caution: Store in a tightly-closed, light-resistant container in a cool, dry place."

5. An expiration date of one year from the date of manufacture

Your comments are encouraged, and will be welcome.

Conclusion

In summary, we have completed our review of your food additive petition for the use of natamycin in broiler chicken feeds to retard the growth of *Aspergillus parasiticus* for up to 14 days. We find the petition to be satisfactory in establishing the utility of natamycin (in the form of a premix containing calcium carbonate, natamycin, and lactose) in retarding the growth of *Aspergillus parasiticus* in broiler chicken feeds for up to 14 days.

Please review our comments on the proposed label and proposed regulation to determine if you find them to be acceptable. Also, please provide us with language that properly describes how your natamycin premix should be mixed into broiler chicken feed to ensure that the final concentration of natamycin in feed is actually the same as that intended. Our comments are based on information you have provided us so far. You will need to provide us with additional information, as indicated previously in this letter if you prefer to have an approval for natamycin itself. We will review your response to this letter, and use any suggestions you make as deemed most appropriate to initiate the process for publishing the notice of approval in the Federal Register.

Please do not hesitate to write us, or telephone Dr. Henry Ekperigin at 301-827-0174, if you have questions regarding this letter. Refer to FAP 2234 when making such inquiries.

Sincerely,

George Grabef, Ph.D.
Director
Division of Animal Feeds
Center for Veterinary Medicine